

**§ 630.64 Potency test.**

The concentration of live rubella virus shall constitute the measure of potency. The titration shall be performed in a suitable cell culture system, using either the Reference Rubella Virus, Live, or a calibrated equivalent strain as a titration control. The concentration of live rubella virus contained in the vaccine of each lot under test shall be no less than the equivalent of 1,000 TCID<sub>50</sub> of the reference virus per human dose.

**§ 630.65 Test for safety.**

(a) *Tests prior to clarification of vaccine manufactured in duck embryo cell cultures.* Prior to clarification, the following tests shall be performed on each rubella virus pool prepared in duck embryo cell cultures:

(1) *Inoculation of adult mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(1), and the virus pool is satisfactory only if equivalent test results are obtained.

(2) *Inoculation of suckling mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(2), and the virus pool is satisfactory only if equivalent test results are obtained.

(3) *Inoculation of monkey tissue cell cultures.* A rubella virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in § 630.35(a)(3), except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(4) *Inoculation of other cell cultures.* The rubella virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in § 630.35(a)(3), in rhesus or cynomolgus monkey kidney, in chick embryo, duck embryo, and in human cell cultures except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if results equivalent to those in § 630.35(a)(3) are obtained.

(5) *Inoculation of embryonated chicken eggs.* A suspension of each undiluted rubella virus pool shall be tested in the volume and following the procedures prescribed in § 630.35(a)(5) except that

the virus need not be neutralized by antiserum. The virus pool is satisfactory only if there is no evidence of adventitious agents.

(6) *Inoculation of embryonated duck eggs.* A suspension of each undiluted rubella virus pool shall be tested in embryonated duck eggs, following the procedures prescribed in § 630.35(a)(5), except that the virus need not be neutralized by antiserum and the volume of inoculum per egg shall not exceed 1.0 milliliter. The virus pool is satisfactory only if there is no evidence of adventitious agents.

(7) *Bacteriological tests.* In addition to the tests for sterility required pursuant to § 610.12 of this chapter, bacteriological tests shall be performed on each rubella virus pool for the presence of *M. tuberculosis*, both avian and human, by appropriate culture methods. The virus pool is satisfactory only if found negative for *M. tuberculosis*, both avian and human.

(8) *Test for avian leucosis.* The vaccine shall be tested for avian leucosis, in the volume and following the procedures prescribed in § 630.35(a)(8). The cultures are satisfactory for vaccine manufacture if found negative for avian leucosis.

(9) *Inoculation of cell cultures and embryonated eggs after neutralization of the virus with antiserum.* Each of the tests prescribed in paragraphs (a)(3), (4), (5), and (6) of this section shall be carried out also with rubella virus that has been neutralized by the addition of high titer antiserum of nonhuman, nonsimian and nonavian origin except that the volume of virus suspension of each undiluted virus pool tested shall be no less than 5 ml. The rubella antiserum shall have been prepared by using a rubella virus propagated in a cell culture system other than that used for the manufacture of the vaccine under test, and the cell culture system shall be free of extraneous agents which might elicit antibodies that could inhibit growth of any known extraneous agents which might be present in the vaccine under test. These tests may be performed either before or after clarification of the virus. The virus pool is satisfactory only if the results obtained are

equivalent to those required in those subparagraphs.

(b) [Reserved]

(c) *Tests prior to clarification of vaccine manufactured in rabbit renal cell cultures.* Prior to clarification each rubella virus pool prepared in rabbit renal cell cultures shall be tested as follows:

(1) *Inoculation of adult mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(1), and the virus pool is satisfactory only if equivalent test results are obtained.

(2) *Inoculation of suckling mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(2), and the virus pool is satisfactory only if equivalent test results are obtained.

(3) *Inoculation of monkey tissue cell cultures.* A rubella virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in § 630.35(a)(3), except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(4) *Inoculation of other cell cultures.* The tests shall be performed in the volume and following the procedures prescribed in § 630.35(a)(3) in rhesus or cynomolgus monkey kidney tissue, rabbit renal tissue and human tissue cell cultures, except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(5) *Inoculation of embryonated chicken eggs.* A suspension of each undiluted rubella virus pool shall be tested in the volume and following the procedures prescribed in § 630.35(a)(5) except that the virus need not be neutralized by antiserum. The virus pool is satisfactory only if there is no evidence of adventitious agents.

(6) *Inoculation of rabbits.* A minimum of 15 ml. of each virus pool shall be tested by inoculation into at least five healthy rabbits, each weighing 1500–2500 grams. Each rabbit shall be injected intradermally in multiple sites with a total of 1.0 ml. and subcutaneously with 2.0 ml., of the virus pool, and the animals observed

for at least 30 days. Each rabbit that dies after the first 24 hours of the test or is sacrificed because of illness shall be necropsied and the brain and organs removed and examined. The virus pool is satisfactory only if at least 80 percent of the rabbits remain healthy and survive the entire period and if all the rabbits used in the test fail to show lesions of any kind at the sites of inoculation and fail to show evidence of any viral infection.

(7) *Inoculation of guinea pigs.* Each of at least five guinea pigs, each weighing 350–450 grams, shall be inoculated intracerebrally with 0.1 ml. and intraperitoneally with 5 ml. of the undiluted virus pool. The animals shall be observed for at least 42 days. Each animal that dies after the first 24 hours of the test or is sacrificed because of illness, shall be necropsied. All remaining animals shall be sacrificed and necropsied at the end of the observation period. The virus pool is satisfactory only if at least 80 percent of all animals remain healthy and survive the observation period and if all the animals used in the test fail to show evidence of infection with *M. tuberculosis* or any viral infection.

(8) *Bacteriological tests.* In addition to the tests for sterility required pursuant to § 610.12 of this chapter, bacteriological tests shall be performed on each rubella virus pool for the presence of *M. tuberculosis*, human, by appropriate culture methods. The rubella virus pool is satisfactory only if found negative for *M. tuberculosis*, human.

(9) *Tests for adventitious agents.* Each virus pool shall be tested for the presence of such known adventitious agents of rabbits as toxoplasma, encephalitozoon, herpes cuniculi, the vacuolating virus of rabbits, rabbit syncytial virus, myxoviruses and reoviruses. The virus pool is satisfactory only if the results of all tests show no evidence of any extraneous agent attributable to the rabbit renal tissue or the vaccine.

(10) *Inoculation of cell cultures and embryonated eggs after neutralization of the virus with antiserum.* Each of the tests prescribed in paragraphs (c)(3), (4), and (5) of this section shall be carried out also with rubella virus that has been neutralized by the addition of

high titer antiserum of nonhuman, nonsimian and nonrabbit origin following the procedures and in the volume prescribed in paragraph (a)(9) of this section. The virus pool is satisfactory only if the results obtained are equivalent to those required by that paragraph.

(d) *Clarification.* The rubella virus fluids shall be clarified by following the procedures prescribed in § 630.35(c).

[38 FR 32068, Nov. 20, 1973, as amended at 40 FR 11719, Mar. 13, 1975; 40 FR 25813, June 19, 1975]

**§ 630.66 General requirements.**

(a) *Final container tests.* In addition to the tests required pursuant to § 610.14 of this chapter, an immunological and virological identity test shall be performed on the final container if it was not performed on each pool or on the bulk vaccine prior to filling.

(b) *Dose.* These standards are based on an individual human immunizing dose of no less than 1,000 TCID<sub>50</sub> of Rubella Virus Vaccine Live, expressed in terms of the assigned titer of the Reference Rubella Virus, Live.

(c) *Labeling.* In addition to the items required by other applicable labeling provisions of this subchapter, single dose container labeling for vaccine which is not protected against photochemical deterioration shall include a statement cautioning against exposure to light.

(d) *Photochemical deterioration; protection.* Rubella Virus Vaccine Live, in multiple dose containers, shall be protected against photochemical deterioration in accordance with the procedures prescribed in § 630.36(g).

(e) *Samples; protocols; official release.* The following shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892:

(1) For each lot of vaccine:

(i) A protocol, which consists of a summary of the history of the manufacture of the lot, including all results of each test for which test results are requested by the Director, Center for Biologics Evaluation and Research.

(ii) A total of no less than two 25-milliliter volumes, in a frozen state (–60°

C.), of preclarification bulk vaccine containing no preservative or adjuvant.

(iii) A total of no less than 30 containers of the vaccine from each filling of each bulk lot of single-dose containers. A total of no less than six 50-dose containers or ten 10-dose containers of the vaccine from each filling of each bulk lot of multiple-dose containers.

(2) In addition to the requirements of paragraph (e)(1) of this section, whenever a new production seed lot is introduced, or whenever the source of cell culture substrate must be reestablished and recertified, samples consisting of no less than 100 milliliters in 10-milliliter volumes, in a frozen state (–60° C.), of postclarification bulk vaccine containing stabilizer but no preservative or adjuvant, taken from each of 5 consecutive lots of the bulk vaccine.

(3) The product shall not be issued by the manufacturer until written notification of official release of the lot is received from the Director, Center for Biologics Evaluation and Research.

[38 FR 32068, Nov. 20, 1973, as amended at 41 FR 10430, Mar. 11, 1976; 42 FR 27582, May 31, 1977; 49 FR 23834, June 8, 1984; 50 FR 4138, Jan. 29, 1985; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

**Subpart H—Smallpox Vaccine**

**§ 630.70 Smallpox Vaccine.**

(a) *Proper name and definition.* The proper name of this product shall be Smallpox Vaccine, which shall be a preparation of live vaccinia virus obtained from inoculated calves or chicken embryos.

(b) *Strains of virus.* The strain of seed virus used in the manufacture of Smallpox Vaccine shall be identified by historical records including origin and manipulation, and shall meet the sterility test requirements when tested by the procedure prescribed in § 610.12 of this chapter. The strain of seed virus and every third passage shall be tested by a rabbit scarification procedure and shown to maintain its original dermatropic properties. The test procedure is available upon request from the Director, Center for Biologics Evaluation and Research. Any new strain shall be shown not to produce a